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TITLE: Development of 5D3 mAb and USPIO-Based Theranostics for Image-Guided Prostate Cancer Therapy

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CONTRACTING ORGANIZATION: Johns Hopkins University, Baltimore, MD

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14. ABSTRACT Aggressive prostate cancers overexpress prostate-specific membrane antigen (PSMA), and we target PSMA to deliver the drug to cancer cells. We are using new PSMA-targeting 5D3 antibody in this image-guided drug development. This antibody has higher binding capabilities with PSMA than other existing PSMA-targeting antibodies. Ultra-small superparamagnetic iron oxide (USPIO) nanoparticles are biocompatible and have a high loading capacity for molecules. Since USPIO is an MRI contrast agent, drug delivery and tumor uptake can be detected with MRI. Our purpose is to synthesize, characterize, and test this new system in prostate cancer cells and mouse models of human prostate cancer and using the simultaneous PET-MRI imaging system to evaluate the drug delivery to the tumor and early tumor response to the therapy. We have conjugated the surface of the USPIO with a 5D3 mAb. This complex will be loaded with mertansine (DM1), an anti-tubulin drug molecule. These drug delivery systems are called nano-theranostics since this system has both therapeutic and image-guided diagnostic capabilities. During the first year of the project, we conjugated USPIO with 5D3 monoclonal antibody and developed USPIO-5D3 targeted drug delivery platform. It was loaded with DM1 drug molecules and labeled with imaging agents. We have successfully accomplished the development of USPIO-5D3-DM1 nano-theranostics and validate them by in vitro binding affinity studies. In vivo biodistribution study of USPIO-5D3-DM1 in mouse models shows high tumor uptake of nano-theranostics by PSMA(+) tumors by Xenogen in vivo optical imaging. These results were also confirmed by 9.4T MRI showing reduction in T2 contrast. Fine tuning of the antibody and drug loading and optimization are yet to be done.					
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ANNUAL REPORT - YEAR 1

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1. INTRODUCTION

Aggressive prostate cancers overexpress prostate-specific membrane antigen (PSMA), and it can be used as a biomarker to deliver drugs to PSMA(+) cancer cells. Here, we are using new PSMA-targeting 5D3 monoclonal antibody (mAb) in this proposed image-guided drug development. This antibody has higher binding capabilities with PSMA than other existing PSMA-targeting antibodies. Ultra-small superparamagnetic iron oxide (USPIO) nanoparticles are biocompatible and have a high loading capacity for cargo molecules. Since USPIO is an MRI contrast agent, drug delivery and tumor uptake can be detected with MRI. Our purpose is to synthesize, characterize, and test this new drug delivery system in prostate cancer cells and mouse models of human prostate cancer. We will also use the simultaneous PET-MRI imaging system to evaluate the drug delivery to the tumor and early tumor response to therapy. We have "decorated" the surface of the USPIO with a 5D3 mAb. This complex was loaded with mertansine (DM1), an anti-tubulin drug molecule. These drug delivery systems are called nano-theranostics since this system has both therapeutic and image-guided diagnostic capabilities. The proposed studies build on a strong foundation to develop a highly effective and non-invasive image-guided drug delivery system, using biocompatible high capacity USPIO nanoparticles, to treat PSMA-overexpressing prostate cancer. The intellectual discoveries, methodology, and technologies developed as a result of the proposed project will be disseminated in the medical research community throughout the project, thus helping fellow researchers in the field to help with in-progress or future discoveries.

2. KEYWORDS: Anti-PSMA monoclonal antibody, ultra-small superparamagnetic iron oxide nanoparticles (USPIO), prostate cancer, targeted therapy, image-guided therapy, theranostics, *in vivo* molecular imaging

3. ACCOMPLISHMENTS

During the first year of the project, we conjugated USPIO with 5D3 monoclonal antibody and developed USPIO-5D3 targeted drug delivery platform. It was loaded with DM1 drug molecules and labeled with imaging agents. The USPIO-5D3-DM1-Fluor was first validated by *in vitro* imaging and binding affinity studies followed by *in vivo* optical and MR imaging. We have successfully accomplished the development and validation of proposed drug delivery system. Fine tuning of the antibody and drug loading and optimization are yet to be done.

What were the major goals of the project?

Specific Aims of the proposal are:

Specific Aim 1: To develop and optimize USPIO-5D3-DM1 nano-theranostics and evaluate them *in vitro*.
(Timeline: month 1-9)

Specific Aim 2: To determine the pharmacokinetics and biodistribution of USPIO-5D3-DM1 nano-theranostics in PSMA(±) mouse models of human PC.
(Timeline month 10-24)

Specific Aim 3: To determine the toxicology, tumor uptake, and treatment response of USPIO-5D3-DM1 nano-theranostics in PSMA(±) mouse models.
(Timeline: month 25-36)

Specifically for the first year of the project the approved SOW includes the following activities:

Aim 1

Major task 1: Synthesis and characterization of USPIO-5D3-DM1-Fluor (100% completion).

Subtask 1: Synthesis of USPIO-5D3-DM1-Fluor

Subtask 2: Characterization of USPIO-5D3-DM1-Fluor

Major task 2. Validation of USPIO-5D3-DM1-Fluor for binding affinity, internalization and *in vitro* therapeutic efficacy in PSMA(±) PC cells (60% completion).

Subtask 1: Binding affinity and internalization in PSMA(±) PC cells.

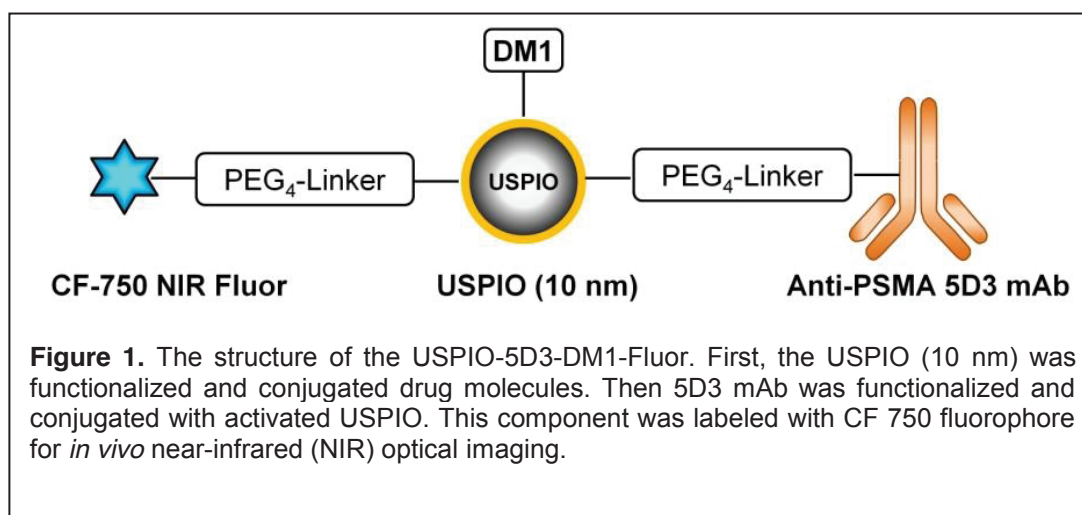
Subtask 2: *In vitro* therapeutic study in PSMA(±) PC cells.

Aim 2: To determine the pharmacokinetics and biodistribution of USPIO-5D3-DM1 nano-theranostics in PSMA(±) mouse models of human PC.

(*In vivo* biodistribution study was started; 20% completion)

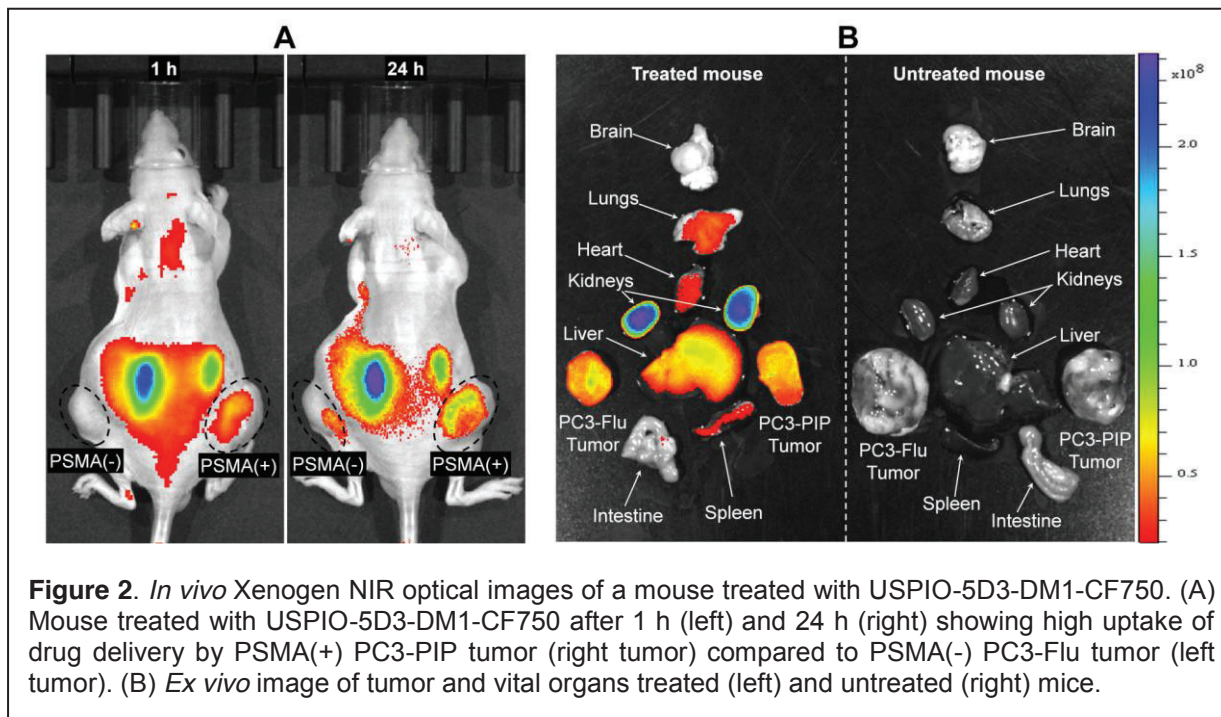
What was accomplished under these goals?

(i) We have successfully synthesized USPIO-5D3-DM1-Fluor and characterized it by DLS and in *in vitro* cellular studies. The drug delivery system was synthesized with high percentage of yield and highly uniform and stable 10 mg batches were produced (Figure 1).



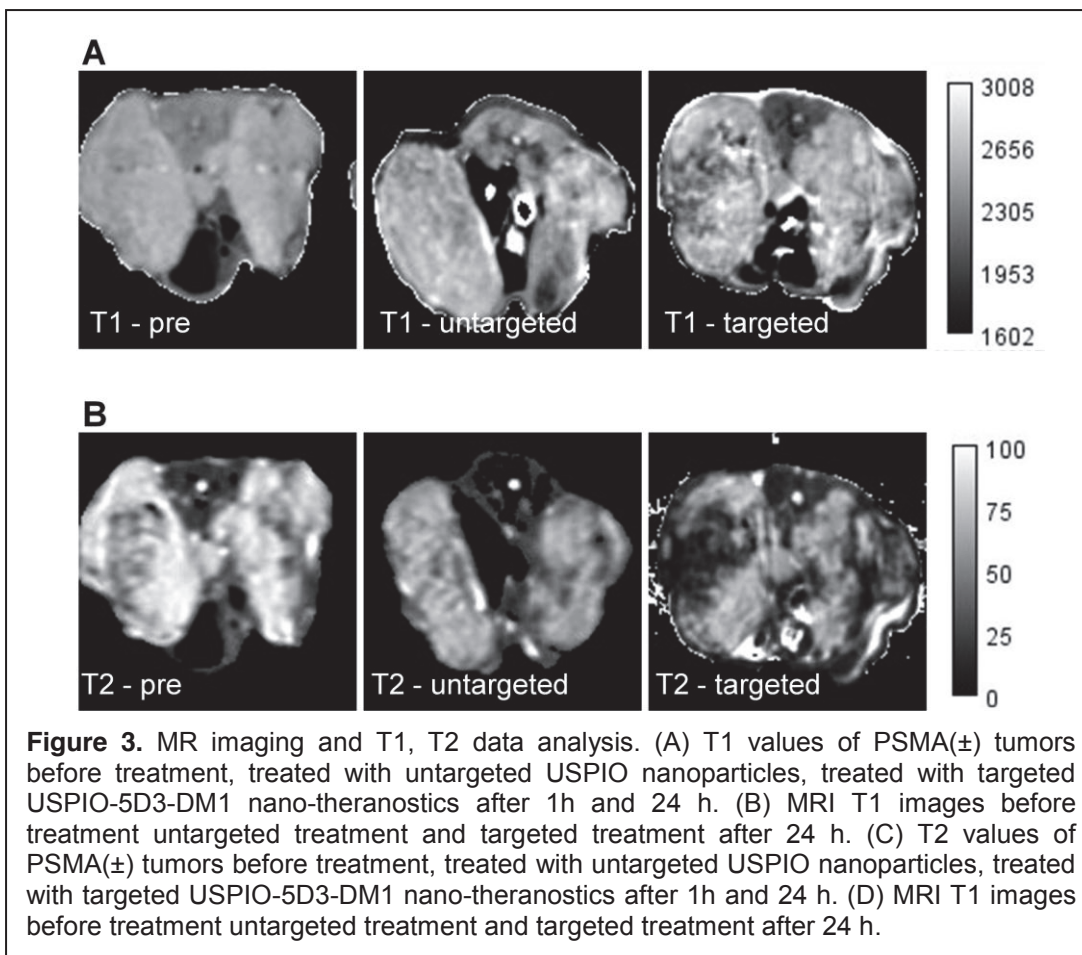
(ii) We conducted experiments to observe the *in vitro* internalization of nano-theranostics in PSMA (+) and PSMA (-) cells. The drug delivery system is internalized fast into the cells that overexpress PSMA on the cell surface. We are currently studying the quantitative binding

affinity experiment by FACS. The preliminary experiments have shown good binding affinity for PSMA(+) PC cells. The *in vitro* therapeutic study in PSMA(±) PC cells is currently in progress. We expect high *in vitro* therapeutic efficacy of USPIO-5D3-DM1-Fluor in PSMA(+) cells without altering the binding affinity.



(iii) The USPIO-5D3-DM1 nano-theranostics was conjugated with CF-750 NIR dye to produce optically detectable USPIO-5D3-DM1-CF750 analogue. Biodistribution and tumor uptake of USPIO-5D3-DM1-CF750 were studied in PSMA(±) tumor mouse models. The PSMA(+) and PSMA (-) dual tumor mouse models were prepared by inoculation of PC3-Flu and PC3-PIP cells on right and left flank of mice respectively. Mice were injected with USPIO-5D3-DM1-CF750 nano-theranostics i.v. and imaged after 1 h and 24 h using Xenogen *in vivo* live animal optical imaging system. The results show high tumor uptake of nano-theranostics in PSMA(+) PC3-PIP tumor (Figure 2A). However; we observed high liver uptake of nano-theranostics and renal excretion of CF-750 via kidneys. After 24 h, mice were euthanized and tumors and vital organs, brain, heart, lungs, liver, kidneys, spleen, and intestine were extracted and imaged *ex vivo* using Xenogen (Figure 2B). We did not see a significant uptake of nano-theranostics in PC3-PIP tumors compared to PC3-Flu tumor in *ex vivo* Xenogen images. We assume that the tumor size and stage difference affect *ex vivo* fluorescence intensities.

(iv) MRI images were taken after systemic i.v. administration of USPIO-5D3-DM1-CF750 in dual tumor mouse models. We observed a significant change in T1 contrast in tumors compared to the pre-scan; however, there was no significant T1 contrast difference between PSMA(+) and PSMA(-) tumors. The T2 images show a considerably higher tumor uptake of USPIO-5D3-DM1 in PSMA(+) tumors compared to PSMA(-) tumor. We are currently determining MR relaxation properties of the nano-theranostics and evaluating significance of the tumor uptake. Our near term plans include repeating MRI experiments using USPIO with different surface functionalities.



What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Scientific presentations were given at laboratory seminars, Cancer Imaging Research Divisional meeting, and Radiology Research Day seminar at the Johns Hopkins School of Medicine.

What do you plan to do during the next reporting period to accomplish the goals?

During the next project period (Year 2), we will synthesize ⁸⁹Zr-labeled analogue of USPIO-5D3-DM1 drug delivery system and will use nuclear imaging and *ex vivo* γ -counting based pharmacokinetics and biodistribution in human prostate cancer mouse models.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

This novel targeted drug delivery system shows low non-specific binding of drugs in healthy tissues reducing systemic toxicity of therapy. USPIO-5D3-DM1-⁸⁹Zr nano-theranostics can be non-invasively detected by translatable PET and MRI for biodistribution and tumor uptake studies, respectively. Therefore, this study would also provide much-needed experimental evidence that the specific and targeted PSMA(+) prostate tumor delivery of USPIO-5D3-DM1 theranostics can effectively suppress tumor growth in preclinical models of primary and

metastatic human prostate cancer with minimal off-target effects and systemic toxicity. The success of our project addresses the overarching challenges to: (a) develop treatments that improve outcomes for men with lethal prostate cancer; and (b) improve the quality of life for survivors of prostate cancer. This research project will be greatly beneficial for the treatment of patients with both primary and metastatic prostate cancer especially for service members and veterans in their 45-60 year age, which is the highest diagnostic age-slot for prostate cancer. The knowledge gained and discoveries produced by this project are disseminated to other researchers, and help them to improve the quality of future research.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change:

We synthesized fluorescent labeled analogue of USPIO-5D3-DM1 drug delivery system and observed the biodistribution in animal models before synthesizing and use of ⁸⁹Zr radiolabeled analogue. Optical imaging based biodistribution results are helpful for the careful design and use of ⁸⁹Zr radiolabeled USPIO-5D3-DM1 for the nuclear imaging based biodistribution and pharmacokinetics studies. This modification does not represent a significant change in the SOW of proposed project.

Actual or anticipated problems or delays and actions or plans to resolve them:

Due to the Covid-19 issues and subsequent lock down there was a delay for synthesis and development of the nano-theranostics. Hence, *in vitro* binding affinity and therapeutic experiments, and the synthesis of ⁸⁹Zr-labeled analogue of the nano-theranostics were delayed. During the incoming year, we are planning to expedite the experiments and complete *in vitro* and continue *in vivo* image-guided therapeutic experiments.

Changes that had a significant impact on expenditures:

Due to the CoViD-19 outbreak, the academia and research activities in the Johns Hopkins School of Medicine have been shut down on March, 2020. Phase 1 restart plan started in mid June, 2020; however, lab activities, and purchases were slowdown during Phase 1 and Phase 2 reopening sessions to keep low lab occupancy at this time. The hiring process was frozen for one year starting June, 2020. Hence, we were unable to hire the Technician as planed in the proposal. Currently, we have identified an excellent candidate; post-hiring process in progress; and a technician will be joining the group in the near future.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Nothing to Report

Significant changes in use or care of human subjects:

Nothing to Report

Significant changes in use or care of vertebrate animals:

Nothing to Report

Significant changes in use of biohazards and/or select agents:

Nothing to Report

6. PRODUCTS

USPIO-5D3-DM1-Cy3 *in vitro* image-guided drug delivery system

USPIO-5D3-DM1-CF-750 *in vivo* image-guided drug delivery system

Publications, conference papers, and presentations

Journal publications:

Nothing to Report

Books or other non-periodical, one-time publications:

Nothing to Report

Other publications, conference papers, and presentations:

Nothing to Report

Website(s) or other Internet site(s):

Nothing to Report

Technologies or techniques:

Nothing to Report

Inventions, patent applications, and/or licenses:

Nothing to Report

Other Products:

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Sudath Hapuarachchige, PhD	Catherine Foss, PhD	Dmitri Artemov, PhD
Project Role:	Principle Investigator (PI)	Co-Investigator	Co-Investigator
Researcher Identifier	0000-0002-1166-8247	0000-0001-8870-5993	dartemo2
Nearest person month worked:	6.0	2.4	1.2
Contribution to Project:	Development of USPIO-5D3-DM1 drug delivery systems. <i>In vitro</i> and <i>in vivo</i> imaging. Supervising and ensuring stable workflow	Development of USPIO-5D3-DM1-DFO and ⁸⁹ Zr radiolabeling.	MRI imaging and image processing
Funding Support:	DoD/Emerson Collective	NIH/NCI	NIH/NCI

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to Report

QUAD CHARTS:

Nothing to Report

9. APPENDICES

Nothing to Report